A HOLOTRICHOUS CILIATE PATHOGENIC TO THEOBALDIA ANNULATA SCHRANK.

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INTRODUCTORY.

On October 11, 1921, a supply of mosquito larvae for class work in the Army School of Hygiene was obtained from a field dyke three miles north-east of Blackpool. On examination I found the catch comprised, in approximate numbers, 100 Anopheles bifurcatus Linnaeus, in the second and third instar; 30 pupae and larvae of Theobaldia annulata Schrank, mostly fourth instar with a few third; and 12 pupae and fourth instar larvae of Culex pipiens L. As the determination of the instar in a struggling larva is usually a lengthy affair, each larva was not examined individually. I contented myself with selecting several specimens representative of each size present and determined the instar of these.

Adult mosquitoes being most required, the pupae and larvae of all varieties were kept together in a large vessel of the original water, aerated from time to time, and a number of imagines of T. annulata and C. pipiens was obtained. During this period the laboratory sergeant found 1 of the Theobaldia larvae dead, and in accordance with custom he removed it for clearing and mounting. On the morning of October 20 I carefully looked over the larvae in the vessel, and satisfied myself that all were alive; there were then about 15 Theobaldia larvae left. About an hour later I noticed that 1 had died in the interval, and removed it for examination. It was a T. annulata in the fourth instar, and under a low power of the microscope I was surprised to see that the eye pigment had entirely disappeared. On looking more closely I saw that the interior
of its head was swarming with ciliates, over 200 being present; the parasites were packed so tightly in each antenna that individual movement was impossible, and only an occasional undulation passed along the mass. Those in the cavity of the head were swimming about freely and actively; the thorax, abdomen and siphon were similarly infected; the gills were free. Altogether the larva contained from 600 to 800 ciliates, and none could be found in the water from which the larva was taken. Next day another *Theobaldia* larva died, and was found to be similarly parasitised; the eyes of this also had been destroyed. I then heard for the first time of the larva which the serjeant had found dead, and which had reached oil of cloves the previous day. Examination showed this larva to have been infected also, but the parasites were much degenerated owing to non-fixation.

On October 23, a second batch of larvae was obtained from the same dyke, and consisted of 96 *A. bifurcatus* in the second and third instar, and 57 *T. annulata* mainly third and fourth, with an occasional second, instar. These were kept separate from the first batch, but treated in the same manner, and subsequently proved to be infected also.

Both batches of larvae were watched closely for further cases of infection, living larvae being examined microscopically from time to time, particular attention being paid to any which seemed sluggish or otherwise abnormal, and an occasional larva was dissected and examined for parasites.

Microscopical examination cannot be relied on to detect a light infection, especially in a living larva.

Two *Theobaldia* pupae of the original batch died on November 7. These were also infected, but to a much less degree than the larvae, which presumably explains their reaching the pupal stage. The chitinous tubes within which the legs form contained ciliates, which were also present inside the abdomen of one imago. This precludes any possibility of a post-mortem invasion, apart from the fact that the exo-skeleton was quite undamaged and that the pupae were only recently dead. One, indeed, had been dead only a few minutes; it came to the surface and straightened itself out and I watched to see the imago emerge; but all movement ceased, and on examination of the pupa I found it was dead and in the condition described.

Subsequently infected larvae and pupae were found as follows:—

<table>
<thead>
<tr>
<th>Type of Larva</th>
<th>Number</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>One (4th instar)</td>
<td></td>
<td>November 7</td>
</tr>
<tr>
<td>Five (one 2nd, three 3rd and one 4th, instar)</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>One (2nd instar)</td>
<td></td>
<td>13</td>
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<tr>
<td>Two (3rd and 4th instar)</td>
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<td>15</td>
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<tr>
<td>Two (4th instar)</td>
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<td>27</td>
</tr>
<tr>
<td>One (4th instar)</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>One pupa</td>
<td></td>
<td>December 1</td>
</tr>
<tr>
<td>One pupa</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Two (4th instar)</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>One (4th instar)</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>One (4th instar)</td>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>
Altogether 22 *T. annulata* were found parasitised; 2 of these were alive when detected but died later; 18 were recently dead and undamaged, and 2 had been dead for several days when discovered. For some reason, these 2 died at the bottom of the vessel amongst débris, and were not noticed at the time; the others died on the surface and were quickly detected as the larvae were kept under close observation and examined several times daily.

The destruction of the eyes already mentioned was a constant feature in the infected larvae, and this was so foreign to my experience of larval disease that an examination of the eye condition constituted the first test of suspected infection, and in no case did a larva with normal eyes prove to be parasitised on further investigation. In one case the ciliates were watched attacking the eyes and were surrounded by whirling clouds of pigment kept in motion by their cilia.

It will be observed that the 22 cases of infection were confined to *T. annulata*, the other mosquito larvae (*A. bifurcatus* and *C. pipiens*) remaining free from invasion by ciliates although kept in the same vessel as the *Theobaldia*. Several larvae died of fungus infection, and several, as larvae do, from no obvious cause, but none of these was found parasitised, either on preliminary examination, or on subsequent dissection which was always performed. *Chironomus* larvae, and a number of crustaceans were also dissected but no sign of infection could be discovered.

None of these ciliates was found at any time otherwise than in association with infected larvae and pupae; an undamaged and recently dead larva might contain 1,000 ciliates but none was ever discovered on examining several watch-glassfuls of the water from which the larva had been removed.

### Methods of Examination.

The parasites were examined after fixation and staining: by *intra vitam* staining; and unstained. In each method, preparations were made showing the ciliates *in situ*, and also after liberation. The larva from which figs. 1, 4 and 5 were drawn was fixed for twenty minutes in Schaudinn's solution containing five per cent of acetic acid, passed through iodine alcohol into distilled water, stained in Meyer's hæmalum for forty-five minutes and differentiated in one per cent HCl in thirty per cent spirit. After being well washed in tap water the larva was passed through the graded alcohols into oil of cloves and kept there until well cleared, controlling this by frequent examinations under the ½ inch objective.

After a day in xylol the specimen was mounted in Canada balsam, some parts whole, others being carefully teased out in the balsam to liberate the contained parasites.

Some larvae were stained entire, or in parts, by iron hæmatoxylin, which gave a good picture, and other methods of staining and fixation were used with more or less satisfactory results. *Intra vitam* staining
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with neutral red and haematoxylin gave beautiful effects, and if the dilution was sufficiently high the ciliates remained active and could be studied for hours.

Fig. 1.—Head of infected larva of Theobaldia annulata Schrank (post-antennal hairs, and some other details, omitted). x 120.

Description of the Parasite.

A holotrichous ciliate of the suborder Hymenostomata, so far only found in the blood of *T. annulata*. The body varies in shape from elongate oval to broad oval, and is longitudinally striated. There is much difference in size, but most of the forms seen vary in length from 25 to 40 μ, the longest I found being 57 μ. The maximum breadth of well-grown individuals usually lies between 15 and 25 μ. The smallest forms measure 8 or 9 μ in length, and every stage in size between these and the mature
Ciliates may on occasion be seen. Considerable variations in shape are noticed; the rapidly swimming forms are relatively long and narrow, and are concave ventrally and convex dorsally; the more slowly moving individuals are relatively shorter and broader, and more or less barrel-shaped.

Fig. 2.—A small form (= 25 μ) unstained, with long caudal cillum. In some individuals the cytostome is considerably longer than shown here.

When enclosed in a restricted space where movement is difficult they progress by slow, flowing movements, suggesting those of an amoeba, the contour of the body conforming to the surroundings; in squeezing past some obstruction, the creature throws out a clear projection of ecto-
plasm into which the glandular endoplasm flows as the obstruction is passed. Such an individual when freed assumes at once its ordinary shape. The smaller individuals show a long caudal cilium; this tail-like structure is present but much shorter in the forms intermediate in size; and is absent in the largest ciliates.

The nucleus is spherical, and in unfixed specimens usually measures 9 to 12 μ; it is frequently found shrunken after fixation. The micronucleus usually lies close to the nucleus, sometimes indenting its margin, and is usually 1-8 to 3 μ in diameter.
The cytostome is placed antero-laterally, and in the specimens measured it varied from 3·6 to 9 \( \mu \) in length and from 1·8 to 4·5 \( \mu \) in breadth; it is relatively longer in the smaller forms. The cytostome is surrounded by circumoral cilia, which are longer in the young individuals; and externally it is provided with an undulating membrane to which a flagellum is affixed; this sometimes appears adherent throughout its course, and at other times the distal portion is free. (See figs. 2 and 3.)

![Diagram of a cell](image)

**Fig. 4:** = 37 \( \mu \). Fixed and stained (Schaudinn and Meyer's hæmalum). The cilia have cleared almost to invisibility. Owing to distortion of the cytostome the details cannot be determined. Compare with figs. 2 and 3.

The endoplasm is granular, and usually contains from 1 to 27 food vacuoles. There is a contractile vacuole posteriorly, pulsating at half-minute intervals at room temperature. There is no anus, food remnants being extruded through the cortex.

When the exo-skeleton of an infected larva is ruptured ciliates dash through the breach and swim about most actively, tending to slow down later, but unless the body is very badly damaged many motile ciliates remain inside. One such larva kept under observation in a watch-glass still showed active ciliates after twenty-six days, whereas the ciliates which had been freed in large numbers could not be found alive after fourteen days, though their disintegrating bodies were plentiful. No sign of encystment was observed.

A word of explanation is necessary here regarding the drawings illustrating this article. As there were no facilities at the Army School of
Hygiene for exact work of this nature, I forwarded stained preparations and living ciliates to the Wellcome Bureau of Scientific Research where Dr. Wenyon most kindly supervised the preparation of these figures. Figs. 1, 4 and 5 were drawn from the former; figs. 2 and 3 from the latter.

**FIG. 5.** = 45-7 μ. Binary fission: fixed and stained (Schaudinn and Meyer's haemalum).

**REPRODUCTION.**

Reproduction takes place by binary fission, the products of division being of equal size and spherical at first, later becoming elongated. In the specimens observed, the lateral indentations of the parent form were obvious before the stage of micronuclear rest was reached, and in one instance these indentations were distinct before the nucleus had completed its elongation. Small forms about 8 μ in length were sometimes present in large numbers, both within infected larvae and after liberation. At other times these small forms could not be found. I supposed that these resulted from repeated divisions, but on several occasions I observed what might be an example of unequal gemmation. One adult about 30 μ in length budded off from its ventral surface a spherical body which at first was attached by a pedicle; this slowly lengthened and the new form was towed behind the parent, the interval gradually increasing until the pedicle ruptured. The new body was spherical and measured 9-4 μ, the nucleus being 4 μ in diameter.
It resembled a product of ordinary binary fission, as seen under similar conditions, except for the difference in size; the process of formation occupied half an hour. I observed this phenomenon twice in one day, but I watched for over a hundred hours before seeing it again. On this latter occasion I removed a pupal fin containing 8 well grown ciliates. They were unable to escape from the fin, and owing to its transparency they could easily be observed. I searched the fin with all powers of the microscope for an hour and a half without finding any bodies other than the 8 large ciliates. After this period several small forms, 9 to 10 μ in length, appeared swimming about inside the fin, and several of the ciliates were watched apparently budding these off; finally the small forms were more numerous within the fin than the large ones. There were no ciliates nor other motile Protozoa in the water in which the fin was mounted. Such a form of multiplication—if my interpretation of this observation is correct—seems extraordinary, though various types of unequal gemmation have been recorded as occurring in other ciliates, *Anoplophrya modulata*, *Hoplitophrya*, *Opalinopsis*, etc.

Nothing resembling an “epidemic of division” was seen, nor could I find any forms in conjugation. It may be recalled that Maupas in his classical studies on *Styloynchia pusulata* observed 215 generations over a period of four months without conjugation.

I did not succeed in finding any indubitable cysts, though ciliates were kept under conditions—desiccation, etc.—which appeared likely to stimulate encystment.

**Possible Method of Infection.**

When liberated ciliates are kept in a watch-glass with a dead, uninfected larva they make no attempt to invade it. I could not make any satisfactory observations on their behaviour to living captive larvae, as the struggles of these scattered any ciliates which approached them. 3 *Theobaldia* larvae kept in small beakers with liberated ciliates did not contract the infection. On the other hand, I examined 15 *Theobaldia* larvae microscopically on several occasions, failing to find any sign of ciliates, and returned them to the original contaminated water as “healthy”; 3 of these eventually died, showing a heavy and obvious invasion.

As regards transmission of parasitic ciliates, Lamborn found that larvae of *Stegomyia scutellaris* kept in water infected with *Lambornella stegomyiae* Keilin, showed infection only after the lapse of three months; also, Brumpt failed to transmit *Anoplophrya brachiarum* Balbiani from infected to healthy *Asellus aquaticus* by keeping them together for one month.

I think that infection of the larva probably occurs by ingestion of small ciliates; these might readily pass through the gut wall into the blood cavity, and so spread over all the body. Large ciliates may be seen
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in various places where they could have entered only when small, for example, inside the bar of the pupal fin.

Position of the Ciliate.

From text descriptions and from the incomplete figure reproduced in Brumpt's "Précis," I thought this ciliate might belong to the genus *Uronema* Dujardin. Wenyon has examined some of my specimens and regards them as possibly belonging to the genus *Cyclidium* Claparede and Lachmann or to the genus *Pleuronema* Dujardin. He points out that *Pleuronema*, as described, differs from *Cyclidium* in having a longer cytostome, and in the absence of a "tail," and that in the ciliate under consideration the small individuals have a long "tail" and a long cytostome, whilst the largest have a relatively short cytostome and no "tail." He makes the very interesting observation that different stages of this ciliate show a transition from long tailed to tailless forms; from a long to a short cytostome; and from long to short peristomal cilia, and that these transitions appear to cut across characters on which a number of different genera have been founded, and that possibly *Uronema*, *Cyclidium*, *Pleuronema*, and *Glaucoma* may all belong to the same genus.

Acknowledgments.

I have already explained my indebtedness to Dr. Wenyon in connection with the drawings for this article; I wish to thank him further, and also Dr. S. H. Daukes, for the great interest and trouble taken in connection with this work.

I am much obliged to Dr. Keilin, of Cambridge University, for examining some of my preparations and confirming my opinion that the ciliate under discussion is not his recently described parasite *Lambornella stegomyiæ* Keilin, and also for the interest he took in this investigation.

References.

Brumpt, E. "Etudes sur les infus. parasit.," Arch. de Parasit., 1912.
Idem., "Précis de Parasit.," 1913.
Lankester, Ray. "Treat. on Zool.," Part I (A. nodulata, etc.).